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13246E_1503_0

Product No. 13246

Fatty Acid Methylation Kit for Glycerides

Features

- This kit is for methyl esterification of fatty acid samples prior to GC analysis.
- The methyl esterification can be performed safely and quickly without heating.
- Suitable for glycerides (e.g. monoglycerides, diglycerides, triglycerides and lecithin).
- Not suitable for free fatty acids, sterol esters and sphingolipids.

Caution: This kit is suitable for measuring fatty acid composition of glycerides. To analyze all fatty acids (except sphingolipids), use the Fatty Acid Methylation Kit (Product No. 06482), by which free fatty acids and sterol esters can also be methylated.

Components

Reagents	Volume	Quantity
A (Solvent)	100 ml	1
B (Reaction Solution)*1	10 ml	1
C (Stop Solution)	100 ml	1

*1 Extreme care must be taken when handling Reagent B (strong alkaline solution).

Required equipment

- Test tube (e.g. φ16.5 mm x 125 mm)
- Pipettes (for quantitative analysis, glass pipettes or positive displacement micropipettes are recommended)
- Vortex mixer

Protocol

1. Put oil sample*2 (below 50mg) into a test tube.
2. Add 1.0 ml of A (Solvent) to the test tube and dissolve the sample.
3. Add 1.0 ml of B (Reaction Solution) and vortex for about 3 seconds.
4. Let the tube stand for about 10 seconds, then add 1.0 ml of C (Stop Solution) and vortex for 5 - 10 seconds.
   (If reaction time is too long, a side reaction occurs, forming free fatty acids. Make sure that the reaction time is less than 1 minute.)
5. After seeing the presence of two layers, transfer upper layer to a new test tube with a pipette.
6. Inject the collected liquid into a GC column.

*2 Extraction of oils and fats from a sample (unnecessary for cooking oil)

(1) Put a sample (e.g. fish meat, beef, potato chips) into a mortar, add acetone, and mash them for several minutes.
(2) Collect acetone solution by filtration or decantation. (Squeeze the residue as much as possible.)
(3) Add hexane (about the same volume as acetone) to the residue, then mash them for several minutes.
(4) Collect hexane solution by filtration or decantation, and mix it with acetone solution. (Squeeze the residue as much as possible. If it is difficult to collect the hexane solution, the next step may be done with residue.)
(5) Add water (about the same volume as acetone) to the mixed solution, then stir it slowly.
(6) Centrifuge it at 1000 - 3000 rpm for 1 – 3 minutes, and collect upper hexane solution.
(7) Remove water by adding anhydrous sodium sulfate (0.2 - 0.3 g per hexane 1ml) and stirring slowly. (It is possible to use this hexane solution (0.2ml or less) as the oil sample.)
(8) Collect hexane solution by filtration or decantation.
(9) Remove hexane by evaporator and get oil and fats.

Caution : The above is a general extraction method of the oil and fats which contain the triglycerides as principal component. The optimum extraction method is different depending on the sample. In quantitative analysis, an internal standard is used. Moreover, repeat extraction is required.

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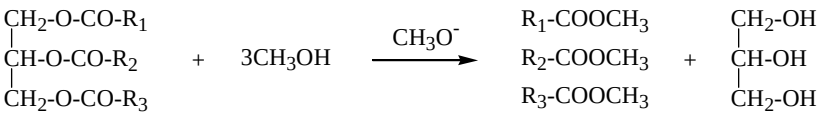
Caution

- Wear protective glasses and gloves.
- When using a vortex mixer, be careful that the liquid does not splash.
- Wash with copious amount of water if your skin is exposed to reagents.
- Reagent B (Reaction Solution) is sensitive to air and deteriorates gradually; use it as soon as possible. Use the supernatant if white precipitate is observed. The precipitate will not affect the methylation reaction.

Reaction mechanism



Storage

Room Temperature

Packing

100 TESTS (Product No.13246-84)

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